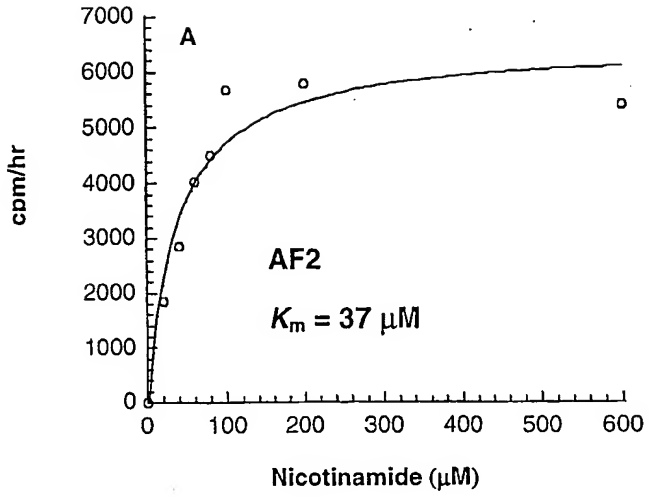
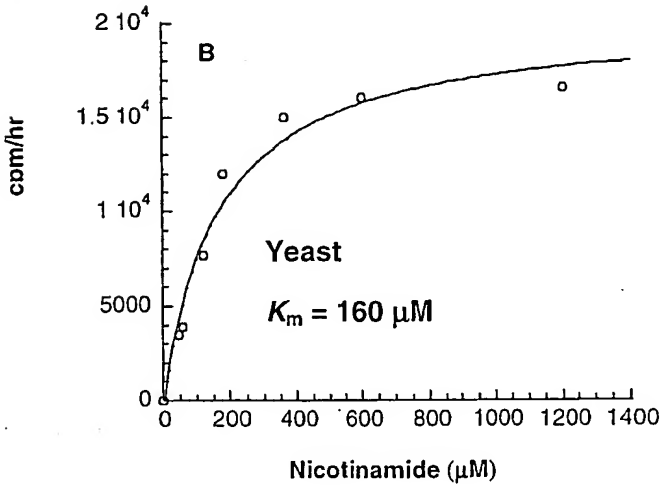
FIG. 1


A
AF2
K_m = 37 µM
cpm/hr
Nicotinamide (µM)
0
1000
2000
3000
4000
5000
6000
7000
0
100
200
300
400
500
600


B
Yeast
K_m = 160 µM
cpm/hr
Nicotinamide (µM)
0
5000
1 10^4
1.5 10^4
2 10^4
0
200
400
600
800
1000
1200
1400

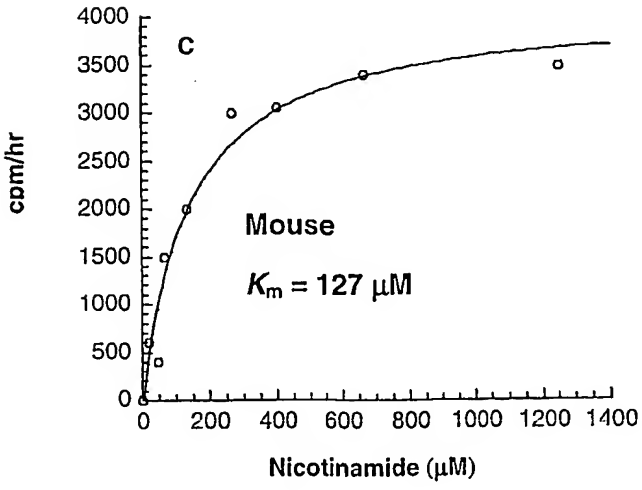

C
Mouse
K_m = 127 µM
cpm/hr
Nicotinamide (µM)
0
500
1000
1500
2000
2500
3000
3500
4000
0
200
400
600
800
1000
1200
1400

FIG. 2

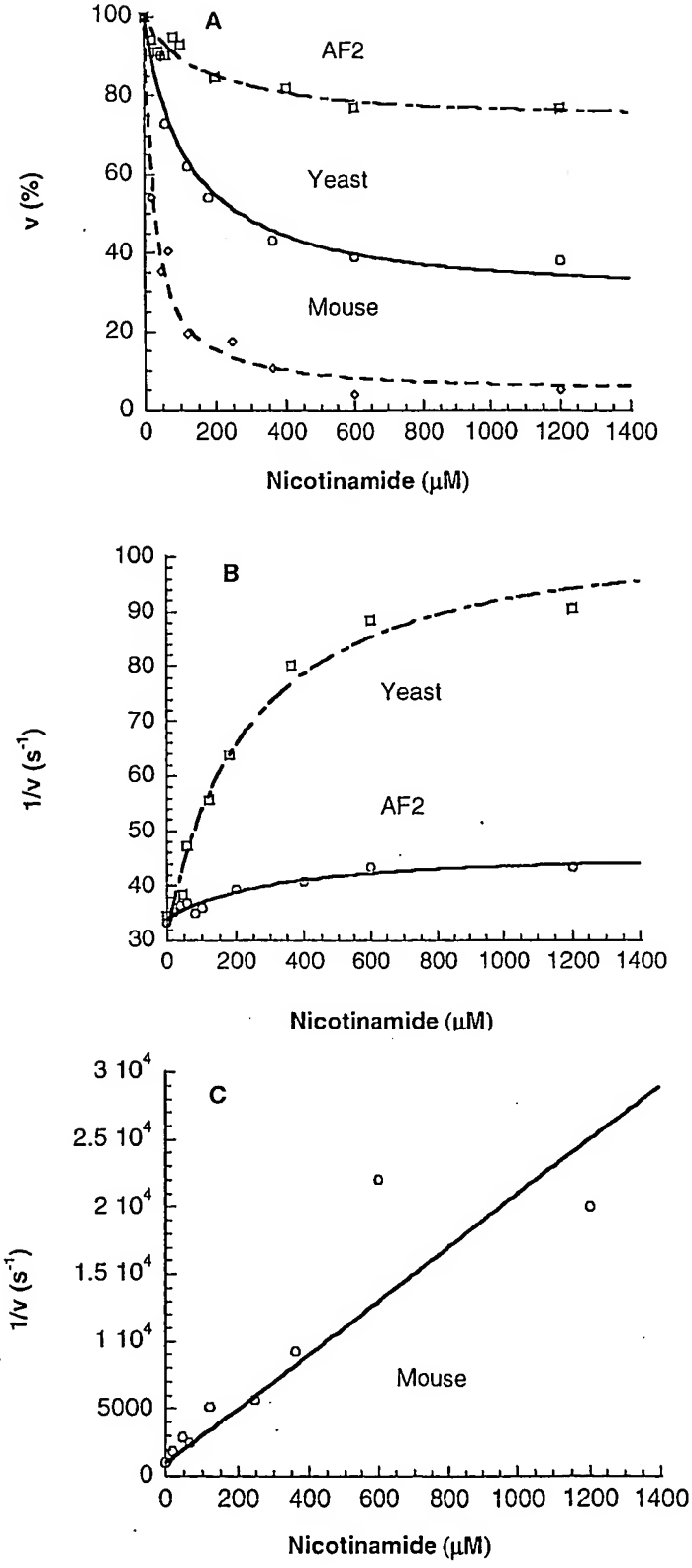

A
AF2
Yeast
Mouse
v (%)
100
80
60
40
20
0
0 200 400 600 800 1000 1200 1400
Nicotinamide (μM)
B
Yeast
AF2
1/v (s-1)
100
90
80
70
60
50
40
30
0 200 400 600 800 1000 1200 1400
Nicotinamide (μM)
C
Mouse
1/v (s-1)
3 10^4
2.5 10^4
2 10^4
1.5 10^4
1 10^4
5000
0
0 200 400 600 800 1000 1200 1400
Nicotinamide (μM)

FIG. 3

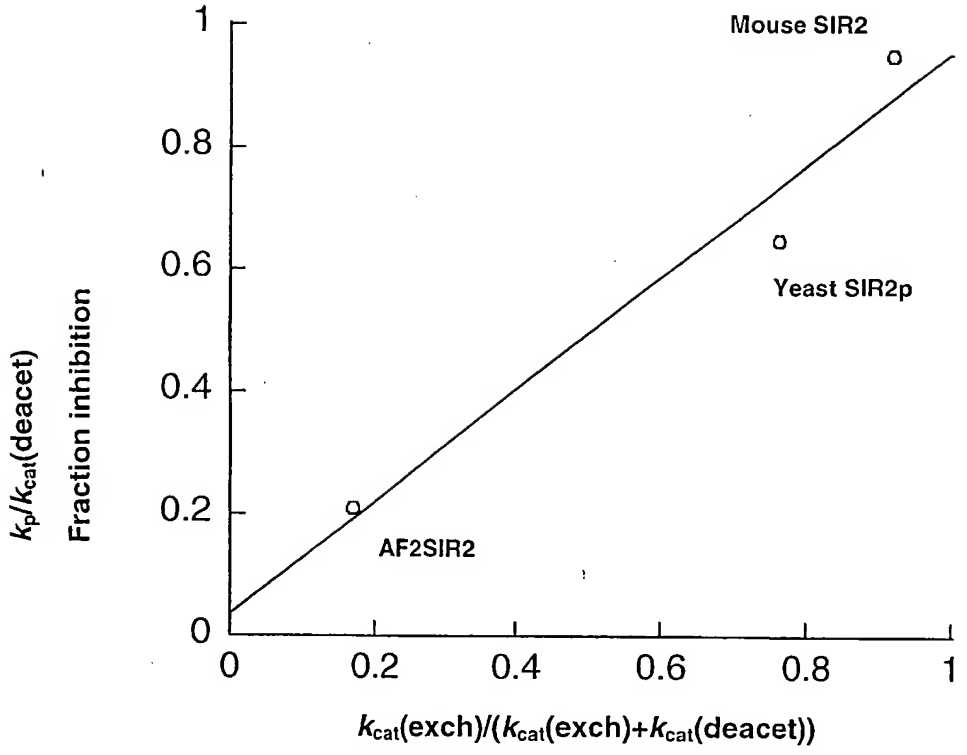

Mouse SIR2
Yeast SIR2p
AF2SIR2
1
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
1
k_p/k_{cat}(deacet)
Fraction inhibition
k_{cat}(exch)/(k_{cat}(exch)+k_{cat}(deacet))

FIG. 4

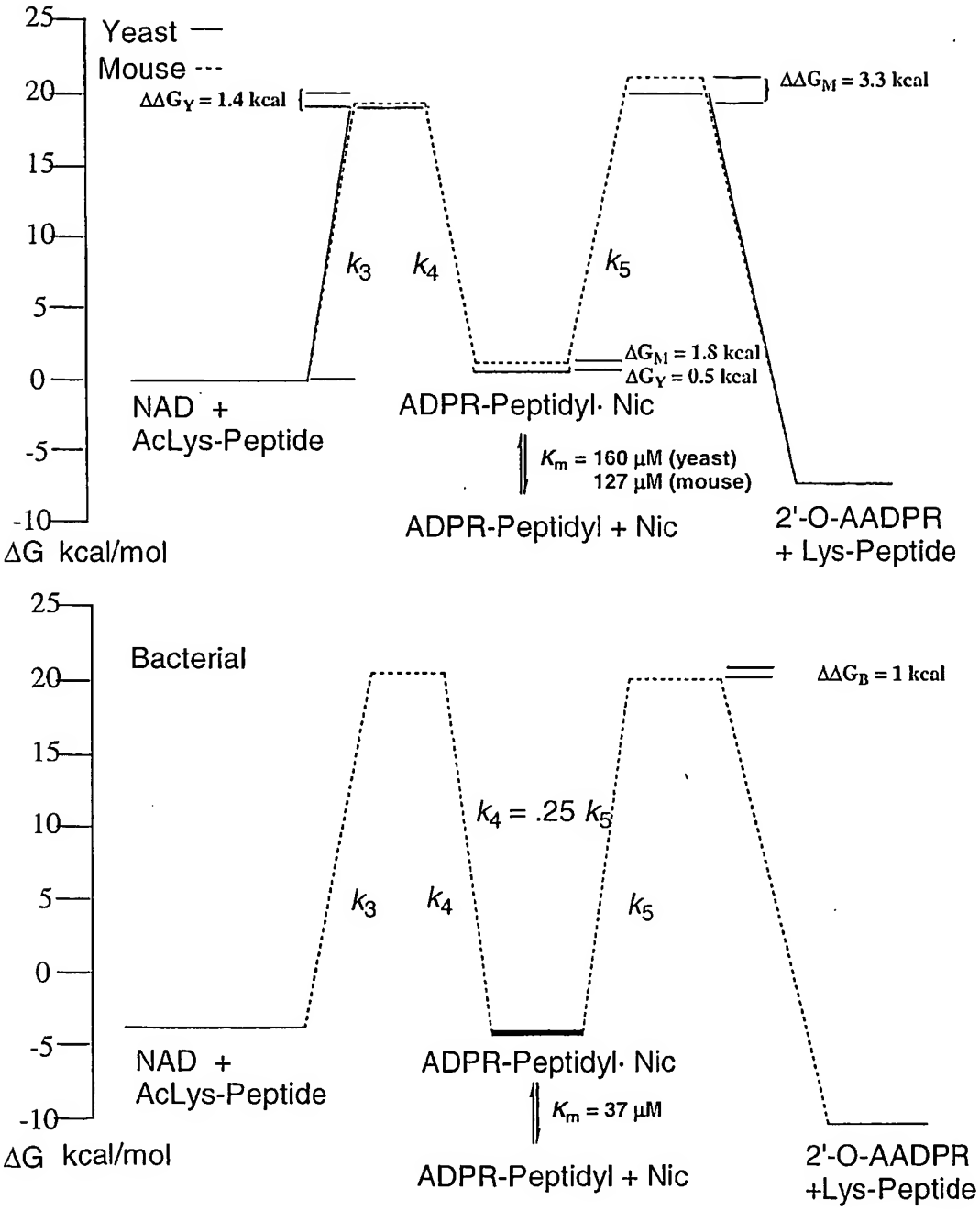

25
20
15
10
5
0
-5
-10
ΔG kcal/mol
Yeast —
Mouse ---
ΔΔG_Y = 1.4 kcal
ΔΔG_M = 3.3 kcal
k3
k4
k5
ΔG_M = 1.8 kcal
ΔG_Y = 0.5 kcal
NAD +
AcLys-Peptide
ADPR-Peptidyl· Nic
K_m = 160 μM (yeast)
127 μM (mouse)
ADPR-Peptidyl + Nic
2'-O-AADPR
+ Lys-Peptide
Bacterial
ΔΔG_B = 1 kcal
k4 = .25 k5
k3
k4
k5
NAD +
AcLys-Peptide
ADPR-Peptidyl· Nic
K_m = 37 μM
ADPR-Peptidyl + Nic
2'-O-AADPR
+Lys-Peptide

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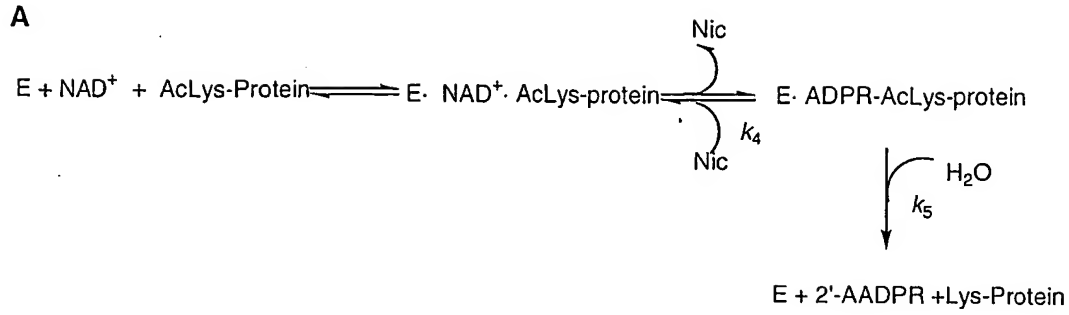
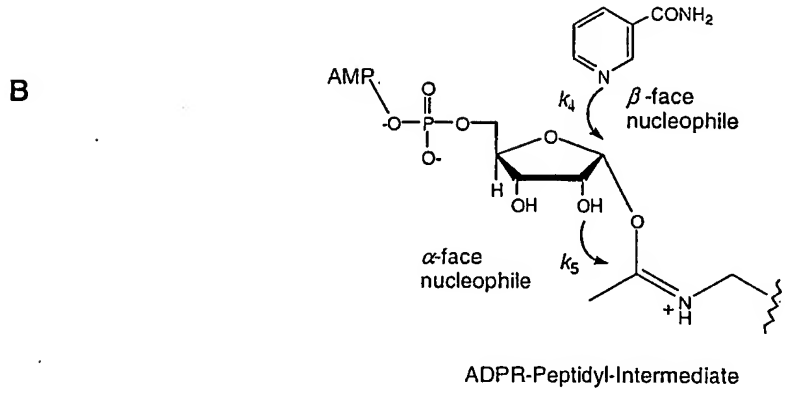
FIG. 5

SCHEME I





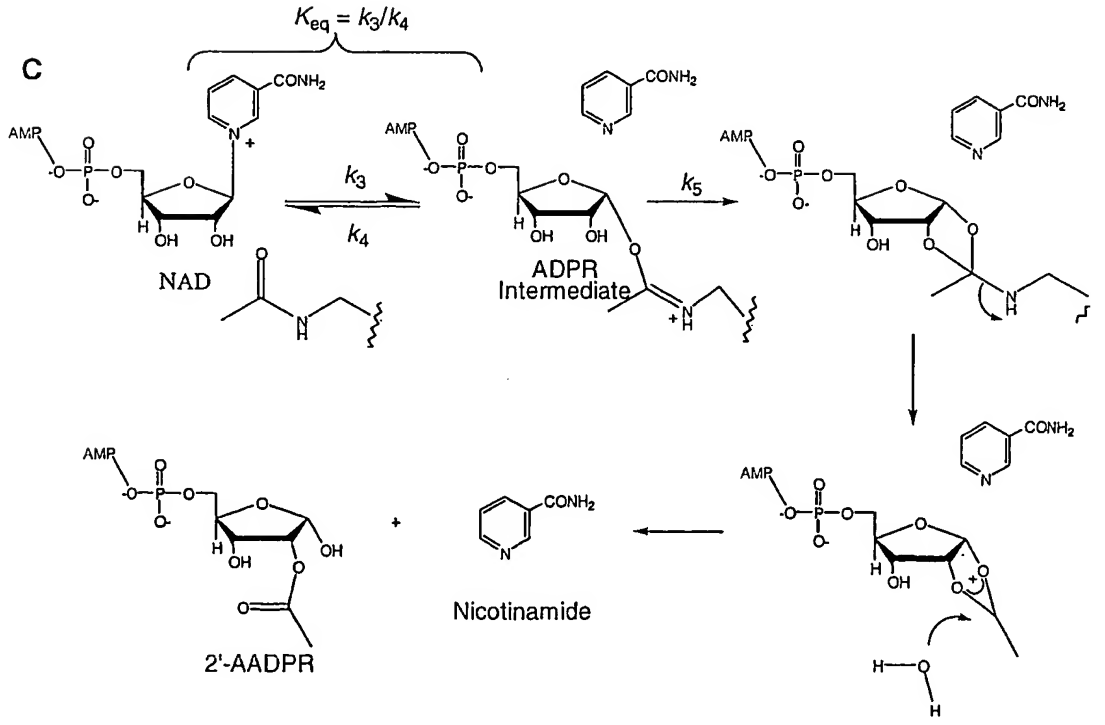


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FIG. 6

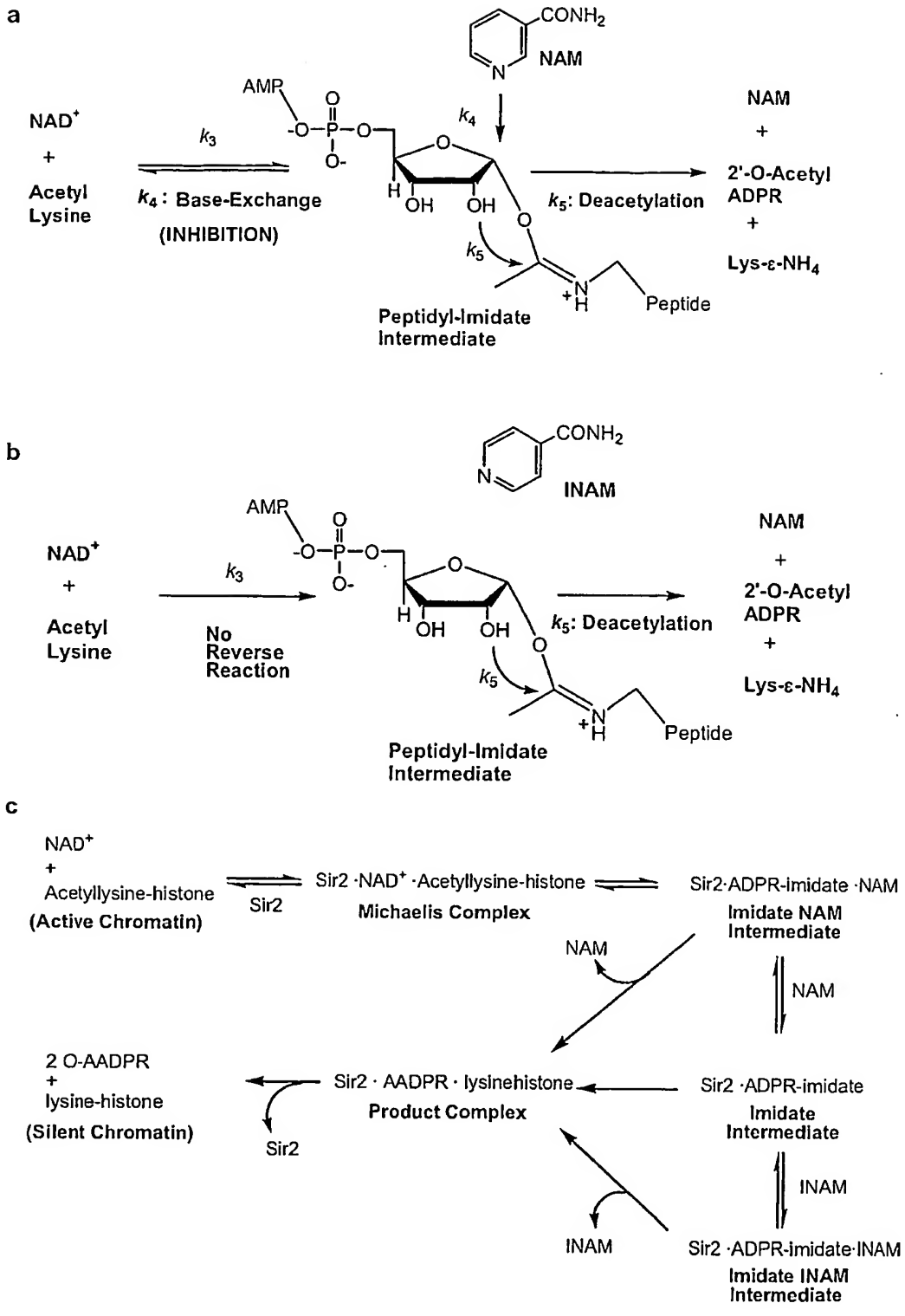
a
NAD+
+
Acetyl Lysine
k3
k4 : Base-Exchange
(INHIBITION)
AMP
CONH2
NAM
k4
k5: Deacetylation
NAM
+
2'-O-Acetyl ADPR
+
Lys-ε-NH4
Peptidyl-Imidate Intermediate
Peptide
b
INAM
NAD+
+
Acetyl Lysine
k3
No Reverse Reaction
k5: Deacetylation
NAM
+
2'-O-Acetyl ADPR
+
Lys-ε-NH4
Peptidyl-Imidate Intermediate
Peptide
c
NAD+
+
Acetyllysine-histone
(Active Chromatin)
Sir2
Sir2 ·NAD+ ·Acetyllysine-histone
Michaelis Complex
Sir2·ADPR-imidate ·NAM
Imidate NAM Intermediate
NAM
NAM
2 O-AADPR
+
lysine-histone
(Silent Chromatin)
Sir2
Sir2 · AADPR · lysinehistone
Product Complex
Sir2 ·ADPR-imidate
Imidate Intermediate
INAM
INAM
Sir2 ·ADPR-imidate·INAM
Imidate INAM Intermediate

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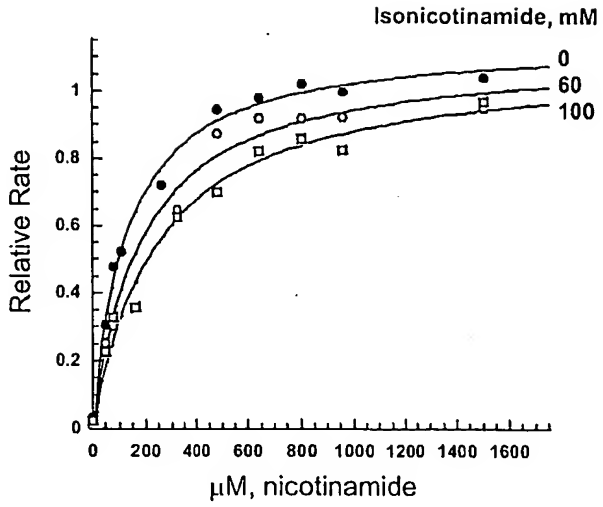
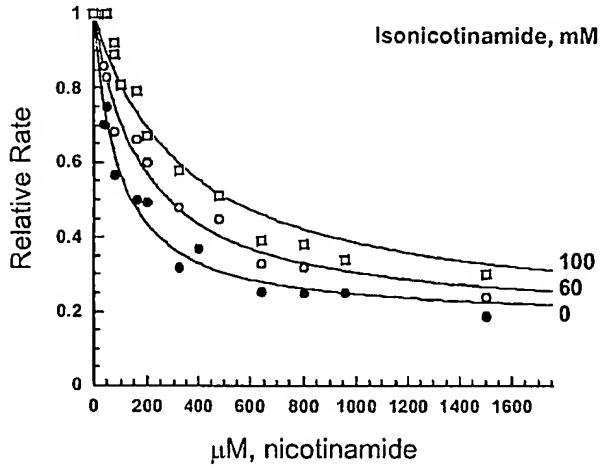
FIG. 7

a


Isonicotinamide, mM
0
60
100
Relative Rate
0
0.2
0.4
0.6
0.8
1
0 200 400 600 800 1000 1200 1400 1600
μM, nicotinamide

b


Isonicotinamide, mM
100
60
0
Relative Rate
0
0.2
0.4
0.6
0.8
1
0 200 400 600 800 1000 1200 1400 1600
μM, nicotinamide

c

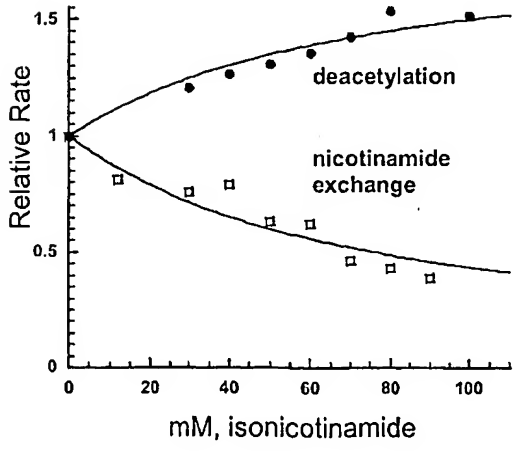

deacetylation
nicotinamide
exchange
Relative Rate
0
0.5
1
1.5
0 20 40 60 80 100
mM, isonicotinamide

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FIG. 8

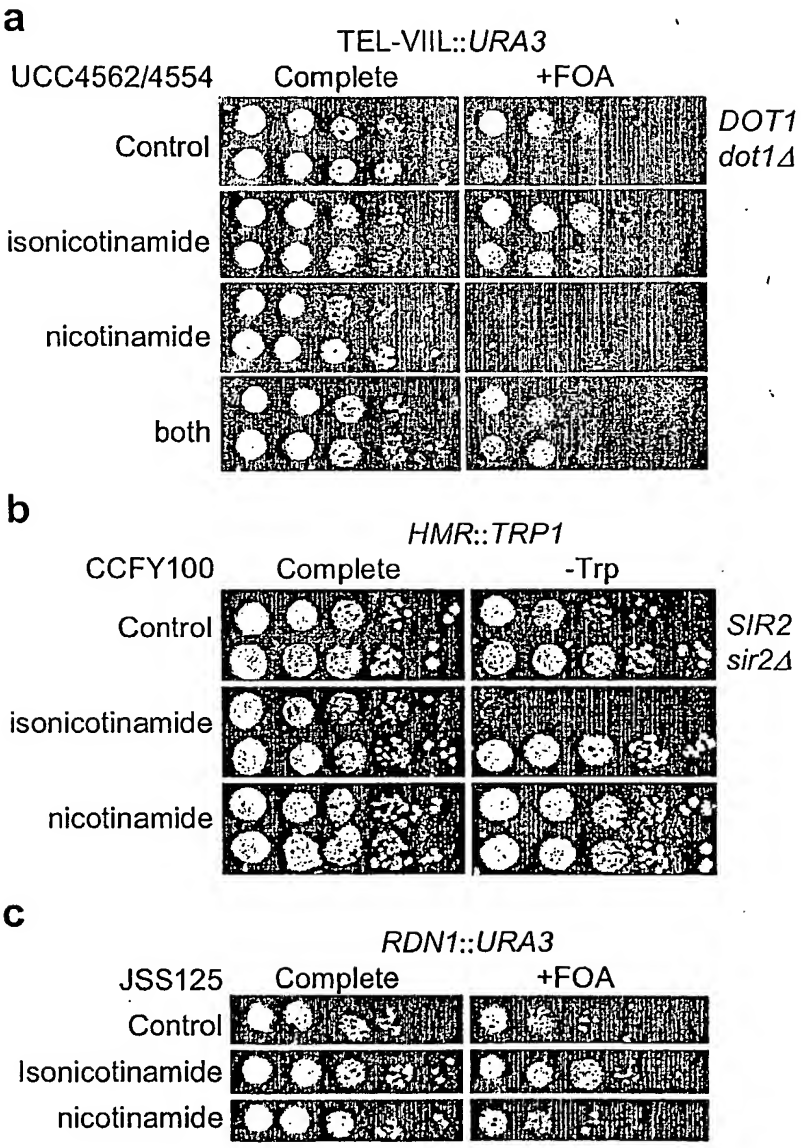

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FIG. 9

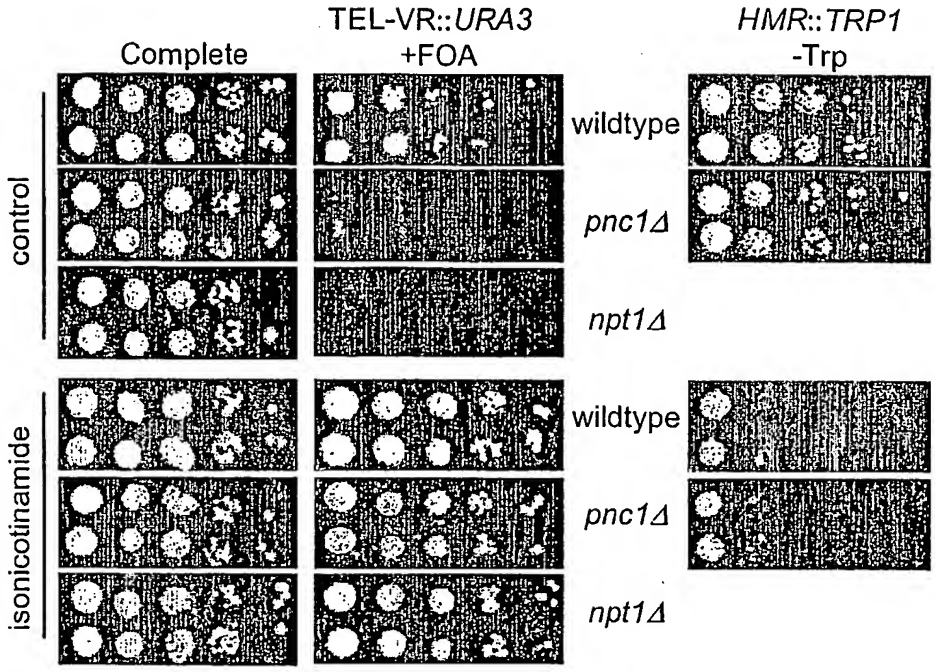
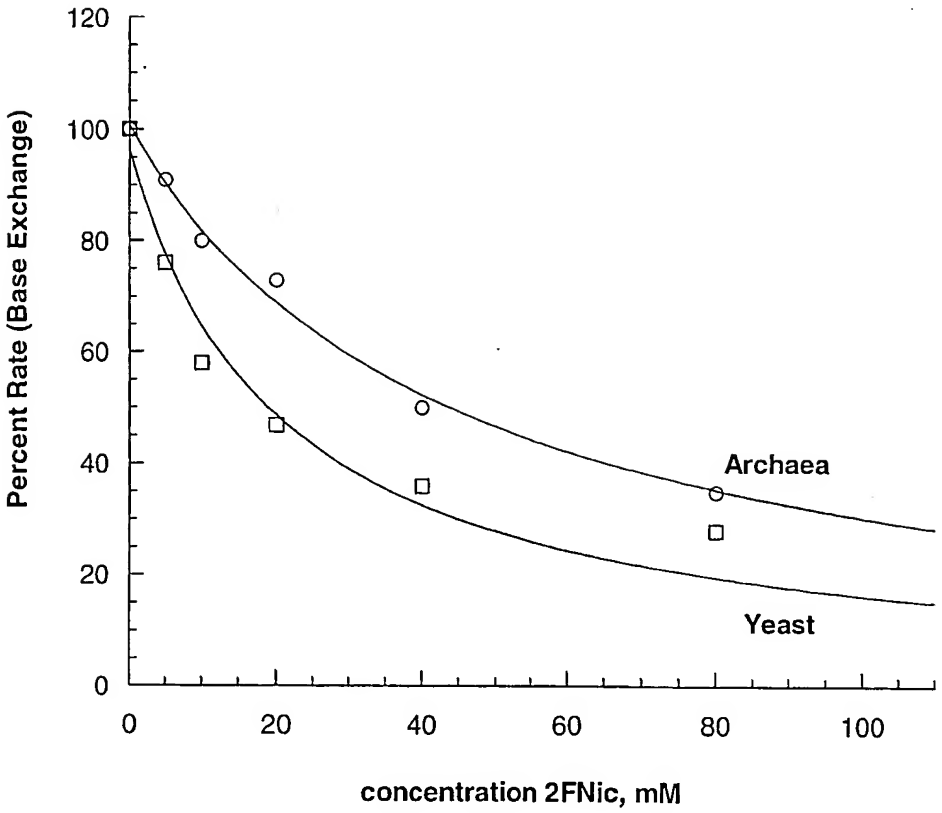


FIG. 10


120
100
80
60
40
20
0
Percent Rate (Base Exchange)
0
20
40
60
80
100
concentration 2FNic, mM
Archaea
Yeast